

AWARD NUMBER: W81XWH-16-1-0665

TITLE: RBPJ and EphrinB2 as Molecular Targets to Treat Brain Arteriovenous Malformation in Notch4-Induced Mouse Model

PRINCIPAL INVESTIGATOR: Rong Wang

CONTRACTING ORGANIZATION: University of California, San Francisco  
San Francisco, CA 94143

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6. AUTHOR(S)  Dr. Rong Wang  E-Mail: rong.wang@ucsf.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  University of California, San Francisco 3333 California Street San Francisco, CA 94143				8. PERFORMING ORGANIZATION REPORT NUMBER	
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14. ABSTRACT We started deleting Rbpj in our P16 mutant mice already developed arteriovenous malformation (AVM) then monitored and analyzed the mice with and without deletion, along with controls at moribund states. We show that endothelial deletion of Rbpj reduced and slowed arteriovenous malformation disease progression, as it doubled the length of time for mutant mice to reach moribundity. We obtained data and performed statistical analysis, showing in all assays for AVM hallmarks, endothelial deletion of Rbpj reduced the severity in these evaluation, which includes whole mount frontal cortex from brain with FITC-lectin+ endothelial cells to highlight vessels; diameters of mutant AV connections; AV shunting using microsphere passage assay; AVM nidus vessels using MICROFIL® casting assay; brain hemorrhage; and regions of hypoxia by Hypoxyprobe™ immunostaining. Our data suggest that deleting Rbpj can induce AVM regression in our mouse model. We are preparing a manuscript to report this finding.					
15. SUBJECT TERMS Brain arteriovenous malformation, arteriovenous malformation, Notch, RBPJ, ephrin-B2, arterial venous specification, vascular, two-photon imaging, mouse, endothelial cells, arteries, veins, dual antiplatelet therapy (DAPT), gamma secretase					
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## 1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

This project addresses the PRMRP Topic Area on *Vascular Malformations*. Our long-term goal is to develop novel therapeutics to ameliorate arteriovenous malformation (AVM), a severe vascular malformation in which arteries carry blood directly to veins through enlarged vessels, known as AV shunts, bypassing capillaries and thus failing to deliver necessary nutrients to tissues. These AV shunts easily rupture, leading to hemorrhage. AVMs pose a high risk to military personnel, as they affect young people, often males, a population highly represented among active military personnel. AVMs can occur anywhere in the body, but brain AVM (BAVM) is the most dangerous form of the disease. BAVMs are only treated by risky neurosurgery or radiotherapy, and potentially lead to life-threatening strokes. The cellular and molecular bases of BAVM pathogenesis remain unknown, limiting the rational design of molecular interventions. A deeper understanding would inspire new discoveries in diagnostic tools, prevention strategies, and pharmacological treatments, improving prognosis and care for patients in both military and civilian populations. Our central hypothesis in this study is that intermediary proteins in the signaling pathway governed by the cell surface protein Notch may serve as new therapeutic targets for BAVM treatment. We and others have linked abnormalities in Notch signaling to AVMs and we have reported that the Notch pathway is hyperactive in BAVM patients. Introducing a constantly active mutant of Notch, called Notch4\*, in mice results in BAVMs in 100% of animals. Built on our strong background and preliminary data, we propose herein to test our hypothesis that inhibition of Rbpj or ephrin-B2, two proteins in the Notch signaling pathway, also causes the regression of Notch4\*-induced BAVMs, demonstrating that BAVMs can be treated by a molecular-level intervention. We also plan to test a drug that has been tested for Alzheimer's disease therapy in humans, DAPT, which inhibits Notch activity. Further, we plan to use state-of-the-art two-photon imaging techniques from our Partner PI's laboratory to visualize disease processes in living animals in real time and provide the first clues on exactly how BAVMs regress after these three interventions. We will improve understanding of the Notch signaling pathway in BAVM prevention and treatment, provide a refined preclinical animal model for the disease, uncover the effectiveness and side effects of vessel specific treatments, offer innovative technical advances in visualizing BAVMs, and shed light on how these treatments lead to the regression of the disease. Given that delivery of treatments selectively to arteries and veins is on the horizon, the proposed research promises new molecular targets and novel vessel-specific therapeutic strategies, ultimately leading to non-invasive treatments, diagnostics, and prevention strategies for BAVM and more broadly for AVMs and other vascular malformations at large.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Brain arteriovenous malformation, arteriovenous malformation, Notch, RBPJ, ephrin-B2, arterial venous specification, vascular, two-photon imaging, mouse, endothelial cells, arteries, veins, dual antiplatelet therapy (DAPT), gamma secretase inhibitor

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

**What were the major goals of the project?**

**List the major goals of the project as stated in the approved SOW.** If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.

<b>Specific Aim 1: Determine the effects of inhibiting <i>Rbpj</i> on the regression of the <i>Notch4</i>* BAVM model.</b>			
<b>Major Task 1:</b> Determine the effect of deleting <i>Rbpj</i> in all endothelial cells on the cessation and regression of <i>Notch4</i> *-induced BAVM	<b>Months</b>	<b>Site 1 PI Wang UCSF</b>	<b>Site 2 Schaffer Cornell</b>
Subtask 1: Generate mutant and control mice for <i>Rbpj</i> deletion using <i>Cdh5</i> (PAC)-CreER <sup>T2</sup>	Continue until publication	Ongoing	
Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation	1-8 mos	More than 90% completion. Require further work until publication of the study.	
Subtask 3: Evaluate data using statistical analysis	6-12 mos	More than 90% completion. Require further work until publication of the study.	
<b>Major Task 2:</b> Determine the effect of deleting <i>Rbpj</i> from the venous and capillary endothelium on the cessation and regression of <i>Notch4</i> *-induced BAVM			
Subtask 1: Generate mutant and control mice for venous <i>Rbpj</i> deletion using <i>Apln</i> -CreER <sup>T2</sup>	Continue until publication	Ongoing	
Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation	5-12 mos	10%	
Subtask 3: Evaluate data using statistical analysis	12-14 mos	Dr. Wang	
<b>Major Task 3:</b> Determine the effect of deleting <i>Rbpj</i> from the arterial endothelium on the cessation and regression of <i>Notch4</i> *-induced BAVM			
Subtask 1: Generate mutant and control mice for	Continue	Ongoing	

arterial <i>Rbpj</i> deletion using BMX(PAC)-CreER <sup>12</sup>	until publication		
Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation	12-20 mos	Dr. Wang	
Subtask 3: Evaluate data using statistical analysis	18-22 mos	Dr. Wang	
<b>Major Task 4:</b> Determine the effect of blocking Notch with DAPT on the cessation and regression of <i>Notch4</i> *-induced BAVM			
Subtask 1: Generate mutant and control mice for <i>Notch4</i> * and treat with DAPT via intraperitoneal injection	Continue until publication	Completed original proposal of IP injection. Confirmed side effects of IP injection that affects the efficacy. Redesigned the delivery route by using oral gavage.	
Subtask 2: Evaluate mice at P36 for hallmarks of BAVM using imaging, histology, and clinical evaluation	16-22 mos	Completed original proposal of IP injection. Confirmed side effects of IP injection that affects the efficacy. Redesigned the delivery route by using oral gavage.	
Subtask 3: Analyze data for attenuation of BAVM symptoms	18-22 mos	Completed original proposal of IP injection. Confirmed side effects of IP injection that affects the efficacy. Redesigned the delivery route by using oral gavage.	
Subtask 4: Evaluate mice after termination of DAPT treatment using imaging, histology, and clinical evaluation for return of BAVM	18-30 mos	Dr. Wang	
Subtask 5: Analyze data for termination effects using statistical analysis	24-30 mos	Dr. Wang	
Milestone: Local IACUC Approval	Prior to project start	Completed	
Milestone: ACURO Approval	4 mos	Completed	
Milestone: Establish effects of <i>Rbpj</i> deletion on BAVM regression	20 mos	Completed, pending further work until publication of the study.	
Milestone: Establish efficacy of DAPT in treatment	30 mos		

of BAVM			
<b>Specific Aim 2: Determine the roles for <i>ephrin-B2</i> as a therapeutic target in the <i>Notch4</i>* BAVM model.</b>			
<b>Major Task 1:</b> Determine the temporal effect of endothelial deletion of <i>ephrin-B2</i> on BAVM progression			
Subtask 1: Generate mutant and control mice for endothelial <i>ephrin-B2</i> deletion using <i>Pdgfb(PAC)-CreER<sup>T2</sup></i>	Continue until publication	Ongoing	
Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation	6-18 mos	20%	
Subtask 3: Evaluate data using statistical analysis	10-18 mos	20%	
<b>Major Task 2:</b> Determine the effect of deleting <i>ephrin-B2</i> in the arterial endothelium on AVM progression			
Subtask 1: Generate mutant and control mice for arterial <i>ephrin-B2</i> deletion using <i>BMX(PAC)-CreER<sup>T2</sup></i>	Continue until publication	Ongoing	
Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation	26-32 mos	Dr. Wang	
Subtask 3: Evaluate data using statistical analysis	30-32 mos	Dr. Wang	
<b>Major Task 3:</b> Determine the effect of deleting <i>ephrin-B2</i> in the venous and capillary endothelium on AVM progression			
Subtask 1: Generate mutant and control mice for venous and capillary <i>ephrin-B2</i> deletion using <i>Apj-CreER<sup>T2</sup></i>	Continue until publication	Ongoing	
Subtask 2: Evaluate mice for hallmarks of BAVM formation using imaging, histology, and clinical evaluation	26-32 mos	Dr. Wang	
Subtask 3: Evaluate data using statistical analysis	30-32 mos	Dr. Wang	
<b>Major Task 4:</b> Determine the effect of deleting <i>ephrin-B2</i> from venous and capillary endothelium on the prevention and treatment of <i>Notch4</i> * mediated BAVMs			
Subtask 1: Generate mutant and control mice for venous and capillary <i>ephrin-B2</i> deletion in <i>Notch4</i> * mice using <i>Apj-CreER<sup>T2</sup></i>	Continue until publication	Ongoing	
Subtask 2: Evaluate mice for BAVM regression using imaging, histology, and clinical evaluation	28-36 mos	Dr. Wang	
Subtask 3: Evaluate data using statistical analysis	34-36 mos	Dr. Wang	
Milestone: Establish effects of <i>ephrin-B2</i> deletion on BAVM regression	36 mos		
<b>Specific Aim 3: Reveal the mechanism of AVM regression by time-lapse live imaging in the <i>Notch4</i>* BAVM model.</b>			
<b>Major Task 1:</b> Imaging cellular dynamics of			

<i>Notch4</i> *-induced BAVM regression after venous and capillary deletion of <i>Rbpj</i>			
Subtask 1: Establish working imaging technique amenable to subsequent 5D imaging	1-12 mos		Dr. Schaffer
Subtask 2: Generate mutant and control mice for venous and capillary <i>Rbpj</i> deletion in <i>Notch4</i> * mice using <i>Apj</i> -CreER <sup>T2</sup>	Continue until publication	Ongoing	
Subtask 3: Use 5D imaging through cranial window to observe cerebrovasculature before and after <i>Rbpj</i> deletion	12-24 mos	Dr. Wang	Dr. Schaffer
Subtask 4: Evaluate data using statistical analysis	18-24 mos		Dr. Schaffer
<b>Major Task 2:</b> Imaging cellular dynamics during BAVM regression after venous and capillary endothelial deletion of <i>ephrin-B2</i>			
Subtask 1: Generate mutant and control mice for venous and capillary <i>ephrin-B2</i> deletion in <i>Notch4</i> * mice using <i>Apj</i> -CreER <sup>T2</sup>	24-36 mos	Dr. Wang (100 mice)	
Subtask 2: Use 5D imaging through cranial window to observe cerebrovasculature before and after <i>ephrin-B2</i> deletion	24-36 mos	Dr. Wang	Dr. Schaffer
Subtask 3: Evaluate data using statistical analysis	30-36 mos		Dr. Schaffer
Milestone: Establish structural and cellular effects of <i>Rbpj</i> deletion on BAVM regression	24 mos		
Milestone: Establish structural and cellular effects of <i>Rbpj</i> deletion on BAVM regression	24 mos		
Milestone: Establish structural and cellular effects of <i>ephrin-B2</i> deletion on BAVM regression	36 mos		

### **What was accomplished under these goals?**

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

**Aim 1: Determine the effects of inhibiting *Rbpj* on the regression of the *Notch4*\* BAVM model.** *We hypothesize that genetic deletion of Notch signaling will lead to the regression of BAVMs.* We propose to determine the effects of deleting Notch signaling mediator *Rbpj* in all endothelium on the regression of the BAVMs (Aim 1.1) and to delineate arterial vs. venous contributions during BAVM regression by deleting *Rbpj* selectively in arterial (Aim 1.2) or venous (Aim 1.3) endothelium. *We also hypothesize that pharmacological inhibition of Notch signaling will lead to the regression of BAVMs.* We proposed to determine the efficacy of Notch inhibitor DAPT on the regression of *Notch4*\*-induced BAVMs by IP injection (Aim 1.4).

***Aim 1.1 Determine the effect of deleting Rbpj in all endothelium on the cessation and regression of Notch4\*-induced BAVM.***

We have made significant progresses on this aim. First, we documented the state of BAVM formation in mice expressing Notch4\* (called Notch4<sup>iGOF-EC</sup> herein). Our data show that expression of constitutive active Notch4 (Notch4\*) in endothelial cells from birth led to features of brain AVM by P16. We obtained data on whole mount frontal cortex from P16 brain with FITC-lectin+ endothelial cells to highlight vessels. We measured diameters of mutant AV connections, which is larger than the capillary diameter seen in P16 control brains, along with statistical analysis. We also detected AV shunting using microsphere passage assay. FITC-microspheres (15 µm diameter, too large to pass normal capillaries) were injected into left common carotid artery and allowed to circulate. Microspheres lodged in capillaries in control P16 brains but microspheres passed through AV shunts in mutant P16 brains and lodged in lungs, demonstrated by along with statistical analysis. We also evaluated the AVM nidus vessels using MICROFIL® casting assay for the P16 brain vasculature. Nidus vessels appeared enlarged and tortuous in mutant and not in control brain. Saline perfused whole brain also showed hemorrhage in mutant brain and not in control. We used Hypoxyprobe<sup>TM</sup> immunostaining and showed regions of hypoxia in mutant brains but not in controls, which is supported by statistical evaluations.

Then, we evaluated the effect of Rbpj deletion from P16 in the Notch4\* mutant mice. We evaluated the overall body weight and show that endothelial deletion of Rbpj permitted increased body weight gain in Notch4\* mutant mice, despite comparable baseline body weights at the time of Rbpj deletion. At P16, the time of TAM injection to induce Rbpj<sup>ΔEC</sup> deletion, body weights of mice in all genotypic cohorts were not significantly different from one another. At the time of moribundity/tissue harvest, Notch4<sup>iGOF-EC</sup>;Rbpj<sup>ΔEC</sup> mice (experimental group) gained significantly more weight than Notch4<sup>iGOF-EC</sup> mice and Notch4<sup>iGOF-EC</sup>;Rbpj<sup>ΔEC-het</sup> mice, even though they did not reach weight gain of negative control mice. In summary, endothelial deletion of Rbpj allowed the mice to grow better.

Endothelial deletion of Rbpj also doubled the length of time for mutant mice to moribundity. We recorded numbers of subjects at risk at 0, 25, 50, 75, 100 days old and use the number to generate Kaplan-Meier curve. We obtained Kaplan-Meier analysis data showed that time to moribundity doubled in Notch4<sup>iGOF-EC</sup>;Rbpj<sup>ΔEC</sup> mice, as compared to Notch4<sup>iGOF-EC</sup> mice. Control Notch4<sup>iGOF-EC</sup>;Rbpj<sup>ΔEC-het</sup> mice did not increased time to moribundity, as compared to Notch4<sup>iGOF-EC</sup> mice. Rbpj<sup>ΔEC</sup> mice increased time to moribundity but did not match the time of negative control mice.

Significantly, we show that endothelial deletion of Rbpj reversed brain AV shunting and vessel tortuosity in Notch4<sup>iGOF-EC</sup> mice. We performed whole mount frontal cortex imaging for FITC-lectin+ endothelial cells to highlight vessels. We performed all the assays that listed above for the P16 Notch4<sup>iGOF-EC</sup> mice and we found that Notch4<sup>iGOF-EC</sup>;Rbpj<sup>ΔEC</sup> mice were improved under these evaluations. We are currently performing arterial and venous molecular marker analysis to determine the arterial venous programming as a result of Rbpj deletion. We are preparing a manuscript to report these findings.

***Aim 1.4 Determine the effect of blocking Notch with DAPT on the cessation and regression of Notch4\*-induced BAVM.***

***Rationale:*** As sustained Notch4\* expression is required to maintain BAVM, we hypothesize that pharmacological inhibition of Notch signaling mitigates Notch4\*-induced BAVM. We will administer DAPT (Calbiochem/Millipore EMD), which inhibits Notch4\* activation and examine effects on the onset

and severity of BAVM in *Notch4*\* mice. We also hypothesize that continued DAPT treatment is not necessary to repress *Notch4*\*-induced BAVM. Since turning on *Notch4*\* after P21 does not lead to BAVM, we reason that after BAVM regression by DAPT treatment, even if *Notch4*\* activity returns without continuing DAPT treatment, BAVM will not re-appear, so long as DAPT treatment is halted after P21. Our pilot experiment demonstrated effective blockage of *Notch4* activation via DAPT, as measured by reduced *Notch4*-ICD and Cx40 expression in ECs (Fig. 9).

We have performed the key experiments as proposed in this subaim, using PI injection to deliver the drug DAPT. However, we did not observe the effect as we expected. To ensure that this outcome is highly reproducible, we have re-evaluated the proposed method and realized that IP injection may not be the best delivery methods in our mice. We are currently taking an alternative approach and repeat the experiments.

### **What opportunities for training and professional development has the project provided?**

We have 4 postdoctoral fellows who have worked on this project in the past year. I provide them with one-on-one training and mentoring. In addition, we have weekly group meeting where one postdoc presents and the entire group discusses and comments on his/her research progress, including experimental design, data interpretation, and future plans. We also have a bi-weekly journal club, where one postdoc presents a leading article and the group discusses and gives input. I also ask postdocs to submit weekly updates and I am available for any questions during the week. I also meet with my postdocs as needed, in which we analyze data, experimental design, and overall progress. I am also readily available as needed beyond these regular meetings. Postdocs are also encouraged to attend campus-wide Research In Progress meetings, retreats, and other forums as well as national and international conferences. Dr. Schaffer and members of his group also provided 2 photon microscopy trainees to my group. UCSF Animal Care and other Core facility, as well as collaborators also provide hands on technical trainings. Together, these engagements provide many opportunities for professional development for our trainees.

### **How were the results disseminated to communities of interest?**

We are preparing a manuscript to report our finding.

### **What do you plan to do during the next reporting period to accomplish the goals?**

We plan to continue the project as proposed and as outlined in the above “achievement” section. Specifically, we have performed the proposed key experiments for DAPT treatment. However, we did not observe the expected effect. To ensure that this outcome is highly reproducible, we have re-evaluated the proposed method. We are currently taking an alternative approach to administer DAPT by oral gavage and repeating the experiments.

**4. IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

**What was the impact on the development of the principal discipline(s) of the project?**

Our data in this period suggest that deleting Rbpj can induce AVM regression in our mouse model of the disease.

**What was the impact on other disciplines?**

Nothing to report

**What was the impact on technology transfer?**

Nothing to report

**What was the impact on society beyond science and technology?**

Nothing to report

**5. CHANGES/PROBLEMS:**

**Changes in approach and reasons for change**

We have performed the proposed key experiments for DAPT treatment. However, we did not observe the expected effect. To ensure that this outcome is highly reproducible, we have re-evaluated the proposed method. We are currently taking an alternative approach to administer DAPT by oral gavage and repeating the experiments.

**Significant changes in use or care of human subjects - NA**

**Significant changes in use or care of vertebrate animals.- No**

**Significant changes in use of biohazards and/or select agents - No**

**6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

**Publications, conference papers, and presentations:**

Nothing to Report

**Website(s) or other Internet site(s):**

Nothing to Report

**Technologies or techniques:**

Nothing to Report

**Inventions, patent applications, and/or licenses :**

Nothing to Report

**Other Products:***Nothing to Report***7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

What individuals have worked on the project?

Name:	Rong Wang
Project Role:	PI
Nearest person month worked:	5
Contribution to Project:	directed the project, trained and supervised other researchers. She closely guided experiments and data analysis. She coordinated efforts with the Partnering PI and ensured achieving proposed goals timely.
Funding Support	NIH/NHLBI

Name:	Feng Cheng
Project Role:	Post Doc
Nearest person month worked:	4
Contribution to Project:	Worked out crucial Notch4 immunostaining, performed mouse vascular perfusion and mouse genetic breeding, provided genotyping, immunostaining, histological analysis, and molecular expertise.
Funding Support	NIH/NHLBI

Name:	Bert Frederick
Project Role:	Post Doc
Nearest person month worked:	9
Contribution to Project:	He carried out the studies of Rbpj and DAPT described in Aim 1, maintained the mouse colony for this project, performed mouse genetic study, examined animal behavior, survival, and cerebrovascular analysis.
Funding Support	NIH/NHLBI

Name:	Shang Li
Project Role:	Post Doc
Nearest person month worked:	2
Contribution to Project:	maintained two-photon microscope, learnt two-photon imaging, including <i>in vivo</i> two-photon cerebrovascular imaging through cranial window, maintained reporter mice and tested reporter imaging, provided cerebrovascular analysis expertise.
Funding Support	NIH/NHLBI

Name:	Weiwei Xiang
Project Role:	Post Doc
Nearest person month worked:	5
Contribution to Project:	Learnt and became an expert on the mouse model of brain AVM, provided a strong foundation for the entire project by obtaining current quantification data on mutant mice in AV shunting formation, generating data on behavior, survival, and other abnormalities. Continue performing DAPT treatment study.
Funding Support	NIH/NHLBI

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Yes, one of Dr. Wang's grants is closed.

Title: Notch signaling in mouse arterial-venous specification

**What other organizations were involved as partners?**

Nothing to Report

## Other Support

**Wang, Rong**

### ACTIVE

Title: Molecular pathogenesis of brain arteriovenous malformation  
Time Commitment: 4.8 calendar months

Role: PI

Supporting Agency:

NIH/NINDS Name of

Contact: Jim Koenig,

Ph.D.

Address: 6001 Executive Boulevard, Room 2107, Rockville, MD

20852 Performance Period: 12/01/2009-03/31/2019

Level of Funding: \$224,718 / year

Goals: The goal of this study is to determine eNOS pathway as a mechanism for the onset and progression of brain arteriovenous malformation in Notch-mediated mouse models of the disease.

- Aim 1: Determine the effect of endothelial Notch4\* on venous endothelial dysfunction and BAVM formation.
- Aim 2: Determine the effect of endothelial Notch deficiency on arterial endothelial dysfunction and BAVM formation.
- Aim 3: Compare Notch and Alk1 mouse mutants in DA and CV development and BAVM formation.

Overlap: None

Title: RBPJ and EphrinB2 as molecular targets to treat brain arteriovenous malformation in Notch4-induced mouse model

Time Commitment: 4.8 calendar months

Role: PI

Supporting Agency:

DOD

Name of Contact:

Catherine Sanchez

Address: 820 Chandler Street Fort Detrick, MD 21702-5014

Performance Period: 09/30/2016-09/29/2019

Level of Funding: \$352,466 / year

Goals: The goal of this study seeks to test if inhibition of RBPJ and Ephrin-B2 can reduce Notch4\*-mediated brain arteriovenous (AV) malformations in mice.

Aim 1: Determine the effects of inhibiting *Rbpj* on the regression of the *Notch4\** BAVM model.

Aim 2: Determine roles for *ephrin-B2* as a therapeutic target in the *Notch4\** BAVM model.

Aim 3: Reveal the mechanism of BAVM regression by time-lapse live imaging in *Notch4\**

mice.

Overlap: None

## **PENDING**

## **PREVIOUS**

Title: Notch signaling in mouse arterial-venous specification

Role: PI

Supporting Agency:

NIH/NHLBI Name of

Contact: Diane Reid,  
MD

Address: 6701 Rockledge Dr., Rockledge II, Room 7160, MSC-7926, Bethesda, MD  
20892-7926 Performance Period: 04/01/2005-02/29/2017

Level of Funding: \$243,787 / year

Goals: The goal of this study is to understand the role of Notch and related pathways in the molecular mechanisms underlying the embryonic development of parallel artery vein pairs in developing early mouse embryos.

- Aim 1: Examine Vascular Endothelial Growth Factor (VEGF)-mediated cell differentiation as a mechanism underlying heterogeneous arterial- and venous-fated ECs in the primordial DA and CV.
- Aim 2: Examine cell segregation as a mechanism to sort venous-fated ECs in the pDA to the pCV.
- Aim 3: Determine the role of Notch signaling in coordinating the development of parallel artery and vein pairs.
- Aim 4: Determine the requirement of endothelial Notch1 and COUP-TFII in AV specification of adult parallel artery and vein pairs.

Overlap: None

Title: Effects of endothelial deletion of Notch in AVM formation Time Commitment: 0.5 calendar months

Role: PI

Supporting Agency: American Heart Association Name of Contact: Susan Mokhtari

Address: 7272 Greenville Ave., Dallas TX, 75231

Performance Period: 07/01/2013-06/30/2016

Level of Funding: \$63,636 / year

Goals: The goal of this study is to evaluate whether mice lacking both Notch1 and Notch4 receptors in the endothelium develop brain arteriovenous malformation.

- Aim 1: Evaluate vascular pathology in mice lacking both Notch1 and Notch4 in the endothelium.
- Aim 2: Evaluate the mechanisms underlying the vascular pathology in mice lacking Notch in the endothelium.

Overlap: None

Title: Molecular pathogenesis of brain arteriovenous malformation

Time Commitment: 1.8 calendar months

Role: PI

Supporting Agency: American Heart Association

Name of Contact: Susan Mokhtari

Address: 7272 Greenville Ave., Dallas TX, 75231

Performance Period: 07/01/10-06/30/12

Level of Funding: \$127,272

Goals: This proposal is designed with the goal of understanding how Notch4\* initiates AVMs in mice.

- Aim 1: Examine capillary enlargement as the first step of AV shunting in Notch4\*-expressing mice.
- Aim 2: Determine whether Notch4\* promotes vessel enlargement by impairing angiogenesis.
- Aim 3: Determine whether VEGF signaling promotes Notch4\*-

induced BAVMs. Overlap: None

Title: Notch enhances arteriogenesis in smoke-related limb ischemia Time Commitment: 1.8 calendar months

Role: PI

Supporting Agency: Tobacco-Related Disease

Research Program Name of Contact: Kamlesh Asotra, Ph.D.

Address: 300 Lakeside Dr., 6<sup>th</sup> Floor, Oakland, CA 94612-3550 Performance Period: 07/01/09-06/30/12

Level of Funding: \$250,000

Goals: The goal of this project is to ascertain if Notch augments arteriogenesis in smoking-related critical limb ischemia.

- Aim 1: To quantify and localize Notch1 expression in mice exposed to cigarette smoke following experimental hindlimb ischemia.
- Aim 2: To determine whether endothelial Notch1 is required for arteriogenesis in the revascularization following experimental hindlimb ischemia.

Overlap: None

## **8. SPECIAL REPORTING REQUIREMENTS : NA**

- COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.
- QUAD CHARTS: If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.

**9. APPENDICES**: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. DO NOT RENUMBER PAGES IN THE APPENDICES.